Author’s response to reviews

Title: Sirtuin1 activator SRT2183 suppresses glioma cell growth involving activation of endoplasmic reticulum stress pathway

Authors:

Tian Ye (15955431292@163.com)
Liwen Wei (1351888128@qq.com)
Ji Shi (mazetiantian@126.com)
Ke Jiang (jiangke.3@163.com)
Huizhe Xu (xuhuizhe1991@126.com)
Lulu Hu (hululu-mk@126.com)
Lingkai Kong (18036641754@163.com)
Ye Zhang (zy712@139.com)
Songshu Meng (ssmeng@dmu.edu.cn)
Haozhe Piao (piaohaozhe@cancerhosp-ln-cmu.com)

Version: 1 Date: 09 Apr 2019

Author’s response to reviews:

Dear Dr. Swapna Chaudhuri,

We are going to submit a revised manuscript (BCAN-D-19-00370) titled “Sirtuin1 activator SRT2183 suppresses glioma cell growth involving activation of endoplasmic reticulum stress pathway” to your journal. We are grateful to the reviewers for their constructive criticisms and have included several new data in the revised MS.

Major changes in figures:

1. New data have been added to the revised Figures 1A, 2B, 2D, 3A, 4B and 5A.
2. New data showing the effects of a pan-caspase inhibitor, Z-VAD-FMK, on SRT2183-induced growth inhibition has been included in new Figure 2E.

Minor changes have been made to the main text to mention the new data.

Responses to critiques (reviewer comments are underlined):

Tan Bibo (Reviewer 1): In this study, author concluded that SRT2183 as a potential new therapeutic strategy in glioma, and author carried many experiments to verify his conclusion. There are some defects that author should explain as followings:

We thank the reviewer for the comments.

Major:

1. Since author consider that SRT2183 as a potential new therapeutic strategy in glioma, author should verify that SRT2183 is harmless to normal cells. Author should provide experiments to detect the effect of SRT2183 to normal cell line (HUVEC, etc.).

As the reviewer suggested, we examined the effects of SRT2183 on a human normal cell line HUVEC. The results showing that SRT2183 substantially inhibited HUVEC growth as in glioma cell lines was added to the revised Figure 1A, thus indicating that SRT2183 might be harmless to human normal cells. We also tested the effects of SRT2183 in human embryo HEK293 cells and obtained nearly the same results (data not shown). Therefore, SRT2183 should be used locally such as intratumor administration.

2. In this study, author found that SRT2183 was involved in proliferation, apoptosis, autophagy, and ER pathway in glioma cells. Then what is the main effect of SRT2183 in glioma cells?

As we did not observe evident cleavage of caspase-3 and PARP in SRT2183-treated LN229 and U87MG cells (Figure 2D) although FACS data showed a portion of SRT2183-treated cells underwent apoptosis (Figure 2C), we reperformed the experiments and additionally treated the cells with Doxorubicin (DOX), an apoptosis inducer, as the positive control. The revised Figure 2D shows that SRT2183 did not induce any cleavage of caspase-3 and PARP, while DOX indeed triggered the cleavage of caspase-3 and PARP. Furthermore, we show the new data demonstrating that pretreatment with the pan-caspase inhibitor Z-VAD-FMK could not block SRT2183-mediated inhibitory effects on glioma cell growth (new Figure 2E) (Results section, line 293, page 13). Therefore, these data suggest that apoptosis is not the main effect of SRT2183 in glioma cells although it appears that a small portion of glioma cells might underwent apoptosis upon SRT2183 treatment. Taken together, the main effect of SRT2183 in
glioma cells is to induce ER stress-dependent growth inhibition. This notion was included in our revised MS (Discuss section, line 418, page 18).

3. Many genes and proteins related to proliferation, apoptosis, autophagy, and NF-κB/STAT3 pathways were all tested in this study. I suggest that author should arrange these results, and verify the effect axis of STR2183 in glioma cells.

The reviewer makes a good point. It is interesting and important to dissect the effect axis of STR2183 in glioma cells. We will fully explore this effect axis in our future study especially when combination with radiation or TMZ therapy.

Minor:

1. In Fig.1A, asterisk (*) should be provided to show the difference.

Done as suggested.

2. In Fig.2B, PCNA and Ki67 should be detected.

We have now included new data in revised Figure 2B showing SRT2183-induced decrease in Ki67 (Results section, line 265, page 12).

3. In Fig.2D, cleaved caspase-3 should be tested.

We additionally treated the cells with Doxorubicin (DOX), an apoptosis inducer, as the positive control and the new data were included in revised Figure 2D (Results section, line 278, page 12).

4. English should be checked carefully.

Done as suggested.

Ilaria Bellezza (Reviewer 2): The Ms by Ye and co-authors is interesting and well written, however to draw the conclusions Authors shoud address the following points:

We thank the reviewer for the comments.
1. pag 13 line 210: since IC50 of SRT2183 is 5 microM, the concentration of 10 microM cannot be defined as subtoxic.

Correction made as suggested (Results section, line 246, page 11).

2. the authors must explain why 2 out of 4 cell lines have been selected for the experiments that follow the experiments reported in fig. 1 and why acetylation of NF-kb and STAT3 have been shown only in one cell line.

We used LN229 and U87MG cell lines for further experiments, as these two cell lines have different background in the expression of some crucial genes regulating survival and apoptosis: LN229 cells with wild type p53 and mutant PTEN, while U87MG cells with mutant p53 and wild type PTEN.

As the reviewer suggested, we determined the acetylation of NF-κB and STAT3 in another cell line: U87MG and similar results to that in LN229 was obtained. We have included the new data in revised Figure 5A (Results section, line 374, page 16).

3. the authors interpreted the increase in Bip, IRE-1alpha, PERK, and phospho-eif2alpha as a mechanism of cell death. However, these proteins are also involved in protective UPR. Indeed, 4-BPA induced effects on cell viability, although reaching statistical significance, seem to be quite far from reaching any biological significance. In order to strength their conclusions, authors need to check the expression of CHOP, a protein involved in ER stress-induced cell death.

As the reviewer suggested, we examined the expression of CHOP in SRT2183-treated glioma cells. We observed evident expression of CHOP induced by SRT2183 in both LN229 and U87MG cells at 24 and 48 h post-treatment. We have included the new data in revised Figure 3A (Results section, line 307, page 13).

4. Page 17 line 372. the authors should discuss more in depth the link between AMPK and Akt/mTOR pathway.

Done as suggested. Please see line 445, page 19.

5. High resolution Figures must be supplied to permit reading

Done as suggested.

I trust that this addresses the reviewers’ concerns and hope that the revised MS is now acceptable for publication in BMC cancer. We appreciate the reviewers’ helpful and constructive comments and thank you for your help.
Sincerely yours

Songshu Meng, Ph D, Professor

Institute of Cancer Stem Cell, Dalian Medical University Cancer Center,
9 Lvshun Road South,  Dalian 116044, China
Tel: 86-411-8611-0496
Fax: 86-411-8611-0496
Email: ssmeng@dmu.edu.cn